

(b) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in § 436.35 of this subchapter.

(6) *Moisture*. Proceed as directed in § 436.201 of this subchapter.

(7) *pH*. Proceed as directed in § 436.202 of this subchapter, using an aqueous

solution containing 10 milligrams per milliliter.

(8) *Crystallinity*. Proceed as directed in § 436.203(a) of this subchapter.

(9) *Absorptivity*. Determine the absorbance of the sample and standard solutions in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams each of the sample and standard in approximately 150 milliliters of distilled water and mix thoroughly. Dilute each to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of each of these solutions to representative 100-milliliter volumetric flasks. Add about 75 milliliters of distilled water and 5.0 milliliters of 5N NaOH to each and then dilute to volume with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100 - m)}$$

where: m = percent moisture in the sample.

(10) *Identity*—(i) *Rolitetracycline*. Place approximately 100 milligrams of the sample to be used in a test tube, add 5 milliliters of 1N NaOH, and heat gently to boiling for about 15 seconds. (The musty, amine-like odor of pyrrolidine is detectable.) Allow to cool to room temperature. A deep burgundy-red color of the clear solution indicates the presence of rolitetracycline.

(ii) *Nitrate identity*. Transfer approximately 1 gram of sample to a 250-milliliter beaker, add 100 milliliters of water, and acidify with 1 milliliter of acetic acid. Heat to boiling and, with constant stirring, add 10 milliliters of a

10-percent solution of nitron (1,4-diphenyl-3,5-endo-anilino-4,5-dihydro-1,2,4-triazole) $C_{20}H_{16}N_4$ ⁷ in 1N acetic acid. Allow to cool. A heavy precipitate indicates the presence of nitrate.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11159, Mar. 17, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.80 Tetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Tetracycline is [4S -

⁷Nitron is available from J. T. Baker Laboratory Chemicals, North Phillipsburg, N.J. 08865.

(4 α ,4 α ,5 α ,6 β ,12 α) - 4 -
(dimethylamino) - 1,4,4a,5,5a,6,11,12a -
octahydro - 3,6,10,12,12a - pentahydroxy
- 6 - methyl - 1,11 - dioxo - 2 -
naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 975 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 13 percent.

(iv) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 3.0 and not more than 7.0.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the tetracycline hydrochloride working standard similarly treated is 108.2 \pm 3.75 percent.

(vi) Its 4-epianhydrotetracycline content is not more than 2.0 percent.

(vii) It is crystalline.

(viii) It passes the identity test for tetracycline.

(2) *Labeling*. In addition to the requirements of §432.5 of this chapter, each package shall bear on its label or labeling the statement "For use only in the manufacture of nonparenteral drugs."

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 60 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Moisture*. Proceed as directed in §436.201 of this chapter.

(4) *pH*. Proceed as directed in §436.202 of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(5) *Absorptivity*. Dissolve approximately 40 milligrams of the sample (as the anhydrous compound), accurately weighed, in 2.0 milliliters of 0.1*N* hydrochloric acid and dilute with distilled water to 250 milliliters. Transfer a 10.0-milliliter aliquot of this solution to a 100-milliliter volumetric flask, add approximately 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH, dilute to volume with water and mix thoroughly. Treat a sample of the tetracycline hydrochloride working standard in the same manner. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \frac{\text{Potency of standard in micrograms per milligram}}{10} \times \frac{10}{100 - m}$$

where: *m* = Percent moisture in the sample.

(6) *4-Epianhydrotetracycline*. Proceed as directed in §436.309 of this chapter.

(7) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

(8) *Identity*. Proceed as directed in §436.308 of this chapter.

[43 FR 11159, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]